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Research progress of Nedd4L in cardiovascular diseases

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Post-translational modifications (PTMs) are a covalent processing process of proteins after translation. Proteins are capable of playing their roles only after being modified, so as to maintain the normal physiological function of cells. As a key modification of protein post-translational modification, ubiquitination is an essential element, which forms an enzyme-linked reaction through ubiquitin-activating enzyme, ubiquitin binding enzyme, and ubiquitin ligase, aiming to regulate the expression level and function of cellular proteins. Nedd4 family is the largest group of ubiquitin ligases, including 9 members, such as Nedd4-1, Nedd4L (Nedd4-2), WWP1, WWP2, ITCH, etc. They could bind to substrate proteins through their WW domain and play a dominant role in the ubiquitination process, and then participate in various pathophysiological processes of cardiovascular diseases (such as hypertension, myocardial hypertrophy, heart failure, etc.). At present, the role of Nedd4L in the cardiovascular field is not fully understood. This review aims to summarize the progress and mechanism of Nedd4L in cardiovascular diseases, and provide potential perspective for the clinical treatment or prevention of related cardiovascular diseases by targeting Nedd4L.

FACTS

- Nedd4L, containing a C2 domain, four WW domains, and a HECT domain, is involved in various pathophysiological processes, and exert distinct influences on various cardiovascular diseases including hypertension, dilated cardiomyopathy, diabetic cardiomyopathy, myocardial infarction, heart failure, etc.
- Nedd4L is a key regulator of epithelial sodium channel (ENaC) activity.
- Targeting Nedd4L might be the hopeful option for target organ protection in patients with hypertension in the future.
- Mutations of the sodium voltage-gated channel alpha-subunit 5 (SCN5A) gene encoding the NaV1.5 protein leads to the occurrence of cardiomyopathy and arrhythmia.

INTRODUCTION

Post-translational modifications (PTMs) of proteins are the covalent addition of functional groups or proteins. The proteolytic cleavage of regulatory subunits, or the degradation of the entire protein could ultimately change the structure, conformation, and physicochemical properties of proteins [1]. This process could further facilitate the functional diversity of modified proteins from the genome level to the proteome. These modifications mainly included phosphorylation [2], acetylation [3], and ubiquitination [4–6]. As we know, the addition of ubiquitin to substrate proteins is called ubiquitination. Ubiquitin [7] is a low molecular weight protein with highly conserved evolution. Yeast and human ubiquitin only differs in three amino acids [8, 9]. Ubiquitination

can mark the target proteins, allow them to be recognized and degraded by the proteasome. Hence, as an important and common form of protein post-translational modification [10, 11], ubiquitination modifications usually occur on lysine residues and thereby regulate the various cellular pathways. Previous studies have mostly focused on upstream proteins such as transcription factors, but the regulatory mechanisms of upstream proteins are complex and there are many factors affecting this process. Research works from our team focused on the most important downstream factors of cellular damage and found that protein ubiquitination modifications played important roles on target organ protection through modulating neuronal precursor cell expression developmentally downregulated 4 (Nedd4) family members [12–14]. The whole process of ubiquitination is formed by a ubiquitin-proteasome system(UPS), which is mainly composed of ubiquitin-activating enzyme (E1), ubiquitin cross-linking enzyme (E2), ubiquitin ligase (E3) and 26S proteasome [15]. The NEDD4 family is one of the most essential groups of E3 ligase [9, 16], which includes neuronal precursor cell expression developmentally downregulated 4-1 (RPF1), neuronal precursor cell expression developmentally downregulated 4-like (Nedd4L/Nedd4-2), ITCH/atropine-1 interacting protein 4 (AIP4) and etc [17–19]. The ubiquitination process is formed by the enzyme-linked reaction consisting of the following three ubiquitinase [20]: 1. In the case of energy supplying by ATP, ubiquitin-activating enzyme E1 could activate ubiquitin molecules; 2. Ubiquitin-activating enzyme E1 then transfers the activated ubiquitin molecules to ubiquitin-conjugating enzyme E2 ; 3. Ubiquitin ligase E3 finally connects the E2-binding ubiquitin to the target protein [21, 22]. Being the most important enzyme in the ubiquitination process, E3 ligase is presented in three families: RING (really interesting new gene) family [23], E3 enzymes with

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HECT domain (homologous to E6-associated protein C-terminus E3 ligase, HECT E3S) [24–26], and the U-box family [27]. HECT E3S can be divided into three subfamilies based on the presence of different amino acid sequence motifs or domains at the N-terminus [25]: (1) Neuronal precursor cell expression developmentally downregulated 4 (Nedd4) subfamily (9 members) [17, 28], containing tryptophan Acid-tryptophan Acid (WW) motif [29]; (2) HERC (HECT and RCC1-like domains) subfamily (6 members), possessing one or more chromosome condensation 1 (RCC1)-like domains (RLDs) [30, 31]; (3) "Other" HECTs (13 members), containing various domains as well [32, 33]. Namely, Nedd4L is a member of the Nedd4 subfamily of HECT E3S of the ubiquitin-proteasome system(UPS) [34]. Nedd4L is located to human chromosome 18q21(ref. [35]), and Nedd4L in human is homologous to mouse Nedd4-2(ref. [36, 37]). Nedd4L is widely expressed during mouse development [38] and is also involved in the pathogenesis of cardiovascular diseases through multiple pathways. It is composed of C2 domain [39], four WW domains [40, 41] and one HECT domain. During the last decades, the incidence of cardiovascular diseases increased dramatically worldwide [42, 43]. In the past few years, the association between Nedd4L and cardiovascular diseases has been focused by many investigators. Results showed that Nedd4L could regulate cardiac function through various pathways and modulate a series of related physiological and pathological processes. In this article, we summarized the recent progress on the role of Nedd4L in various cardiovascular diseases.

NEDD4L AND HYPERTENSION
Interaction of Nedd4L and ENaC and the potential role on sodium homeostasis and blood pressure
Hypertension is a common disease in the cardiovascular field [44–47], and is an important risk factor for stroke, myocardial infarction, heart failure, and kidney damage. Therefore, the pathogenesis of hypertension is a long-standing research focus [48–51]. Accumulating evidence show that, Nedd4L is involved in the formation and development of hypertension at the molecular level. The association between sodium intake, sodium hemostasis in human body and hypertension is well known now. The presence of Nedd4L in the kidney indicates its role and the interaction on sodium reabsorption in the kidney, thus affecting the blood pressure [52, 53]. A previous study demonstrated that reabsorption of Na^+ [54] could be regulated by the Nedd4 family member-- epithelial sodium channel (ENaC) in the kidney, an important regulator of electrolyte balance and fluid stability [55].

ENaC is structurally composed of three subunits (alpha-, beta-, and gamma-), each containing a conserved proline-tyrosine (PY) motif at the C-terminal. Nedd4 protein could bind to these PY motifs on ENaC, and undergo ubiquitination through its WW domain [56]. Activated ENaC is capable of mediating the transportation of Na^+ through the epithelial cells in the kidney, intestine, and lung [38, 57, 58]. Meanwhile, some biochemical and overexpression experiments have shown that Nedd4L could bind to the β- and γ-subunits of ENaC through its PY motif in the C-terminal region, which could then interact with the WW domain of Nedd4L and undergo ubiquitination for degradation [59, 60]. Phosphorylation of Nedd4L by serum and glucocorticoid-regulated kinase 1 (SGK1), for instance, may also regulate its combination with ENaC, then ubiquitinating ENaC. Thus, the interaction between Nedd4L and ENaC might play an important role in sodium homeostasis, and the regulation of blood pressure (BP) [57]. Therefore, it is of importance to further define the role and mechanism of Nedd4L ubiquitination in the protection of target organs in hypertension. It remains unknown if targeting Nedd4L could be the hopeful option for target organ protection of hypertension.

Nedd4L and essential hypertension
Essential hypertension (EH) is common, but the underlying pathogenesis is complex and still not fully understood. Genetic variants are also considered to be one of the causes of EH. In addition, epigenetic, environment and other factors might also be responsible for the pathogenesis of EH [61]. Among them, post-translational histone modifications are considered to be the crucial epigenetic marks associated with EH [62]. At present, more and more members of the Nedd4 family in the ubiquitinated proteasome system, especially Nedd4L, are constantly being explored for their role in EH through both experimental and clinical studies [38, 63, 64]. It is shown that the Nedd4L gene may be a candidate gene that causes high blood pressure in humans. Nedd4L has three isoforms (isoform I, II, and III). Studies found that the interaction of isoform I might interact with other human isoforms and abnormally increase sodium reabsorption [38]. Loffing-Cueni et.al. have found that Nedd4L is highly expressed in aldosterone-sensitive distal nephron (ASDN) near to the collecting duct [65], indicating its role on hypertension. A previous study in African Americans found that the A allele of SNP rs4149601 was associated with increased blood pressure, with the involvement of Nedd4L. The detailed mechanism may be that this allele could reduce the ubiquitination and degradation of epithelial Na^+ channels, resulting in an increase in the density of epithelial sodium channels or prolonged residence time on the cell surface, which will further lead to the increasing of the epithelial sodium transportation, eventually result in hypertension. Russo et.al. also found two Nedd4L SNPs (rss513563 and rs3865418) were associated with hypertension in American whites, and two other Nedd4L SNPs (rs4149589 and rs3865418) were associated with hypertension in Greek whites [66]. Three representative Nedd4L variants (296921-296923delTTG, rs2288774, and rs2288775) were found to be related to EH in the general Kazakh population in a case survey study among Kazakh women, suggesting that the Nedd4L gene variant may be associated with the essential hypertension in Kazakh's women [61].

Nedd4L and salt-sensitive hypertension
The so-called salt-sensitive hypertension refers to increased blood pressure by relatively high-salt intake [47, 67]. Studies have shown that SGK1 may play a role in the pathogenesis of salt-sensitive hypertension, SGK1 phosphorylates and inhibits Nedd4L which is an ubiquitin ligase, thereby failing to reduce the channel expression and stimulate ion channel degradation. The phosphorylation mediated by SGK1 induces the interaction of Nedd4L with members of the 14-3-3 protein family, which in turn disrupts the ubiquitination and degradation of ENaC by Nedd4L [68, 69] (Fig. 1). It can be said that SGK1, functioning as a mediator, indirectly activates ENaC in tubules [70]. In salt-sensitive hypertensive rats, ENaC is abnormally regulated by aldosterone [71], contributing to the initiation and progression of salt-sensitive hypertension [72, 73]. In recent years, accumulating evidence defined the interaction between NEDD4L and ENaC and their role in the regulation of blood pressure. Nedd4L is now considered to be an E3 ubiquitin ligase involved in the process of salt-sensitive hypertension [74]. It is involved in the control of the sodium transporters, which are distinct from the epithelial sodium channels, but also play a determinant role in salt-sensitive hypertension [75]. A previous study showed that conditional Nedd4L knockout mice showed similar blood pressure as control mice under standard diet, but blood pressure was significantly higher in conditional Nedd4L knockout mice than in control mice after 12 days high-Na^+ diet, accompanied by higher urinary calcium. [71] Another study evidenced higher blood pressure in Nedd4L knockout mice even under normal diet, and blood pressure was further increased under a high-salt diet, studies also found that salt-sensitive hypertension in the Nedd4L-C2 (KO) mice
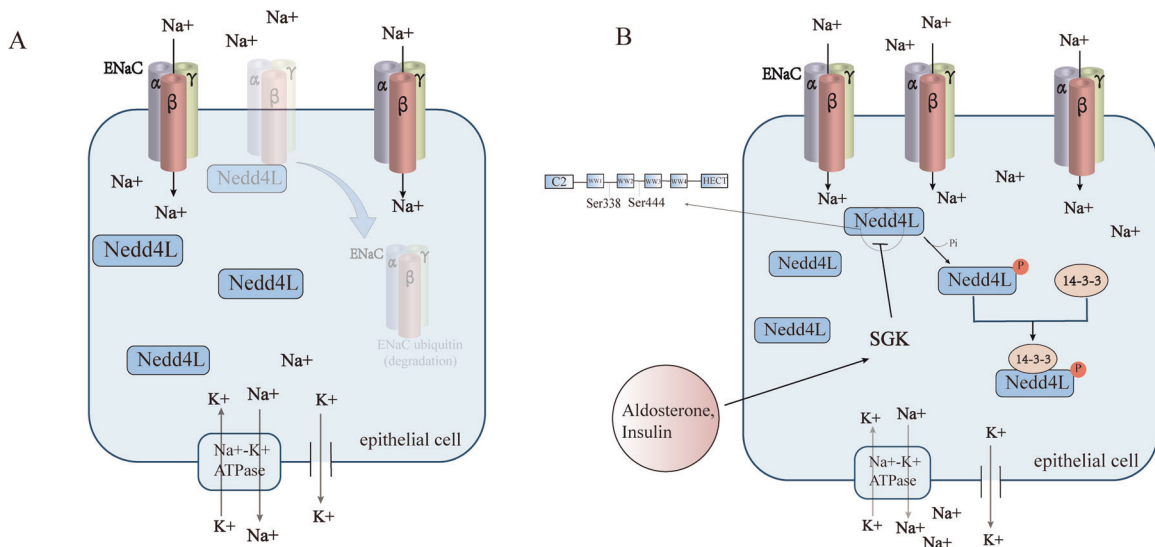
Fig. 1 The mechanism diagrams of how SGK1 regulates sodium reabsorption by Nedd4L. A Epithelial sodium channel (ENaC) can be degraded by Neuronal precursor cell expression developmentally downregulated 4-like (Nedd4L) to maintain sodium balance. **B** Serum and glucocorticoid-regulated kinase 1(SGK1) phosphorylates and inhibits Nedd4L which is an ubiquitin ligase, thereby failing to reduce the channel expression and stimulate ion channel degradation. The phosphorylation (mainly surrounding amino acids Ser444, Ser338) mediated by SGK1 induces the interaction of Nedd4L with members of the 14-3-3 protein family, which in turn disrupts the ubiquitination and degradation of ENaC by Nedd4L, ultimately leading to more sodium reabsorption.

was resistant to eplerenone (EPL) [57]. Aldosterone-independent transcriptional activation of ENaC in the aldosterone-sensitive distal nephron (ASDN) was evidenced in Nedd4L-C2 KO mice, this activation could be abolished by Amiloride [76]. The following factor might be involved in the induction of hypertension in Nedd4L knockout mice: Upregulated expression levels of all three ENaC subunits in the kidneys [57]. However, a specific inhibitor of sodium ion channel, amiloride, can reverse the elevated blood pressure shown in the Nedd4L-KO mice under high-salt diet [76]. The above results indicate that Nedd4L is a key regulator of ENaC activity and blood pressure in vivo. In fact, studies have found that carriers of the Nedd4L salt-sensitivity-associated genotype (rs4149601 A→G and rs2288774 T→C NEDD4L variants) had higher systolic and diastolic blood pressure and multivariate adjusted hazards ratio (95% confidence interval) of CVD 1.13 (1.02-1.25, $P = 0.018$), coronary events 1.20 (1.06-1.37; $P = 0.005$) and cardiovascular mortality 1.17 (0.99-1.37; $P = 0.055$) than noncarriers, suggesting that the genotype of Nedd4L related to salt-sensitivity is associated with hypertension and higher risk of cardiovascular morbidity and mortality in human beings [77]. Nedd4L was once considered as a candidate protein to control the surface expression of ENaC, but which component is indeed involved in the regulation of ENaC in epithelial cells was unknown. Through RNA interference technology, Snyder and colleagues discovered that endogenous Nedd4L could negatively regulate ENaC in epithelial cells, which is the key component of steroid hormone-regulated ENaC signaling pathway, so the defect of this regulation pathway may be directly linked with the pathogenesis of hypertension [78].

Nedd4L and Liddle syndrome
Liddle syndrome is characterized by hypertension with a background of hypokalemia and metabolic alkalosis [79, 80]. It is usually caused by abnormally elevated sodium reabsorption in distal nephrons. The α, β, and γ subunits of ENaC are known to contain a conserved proline-tyrosine (PY) motif at the C-terminus [56]. Mutation in this PY motif, which is also the binding site for the ubiquitin ligase Nedd4L, might cause Liddle syndrome [81, 82] (Fig. 2). In case of β or γ subunits deficiency of the PY motif, binding capacity to the WW domain of Nedd4L will decrease,

thereby accelerate the cell activity of ENaC, promote apoptosis, increase the absorption of sodium ion and fluid in the distal nephron, and ultimately lead to the elevated blood volume and blood pressure [81, 83], implying that Nedd4L may be an inhibitor of epithelial Na^+ channels [84]. Liddle syndrome can be manifested as severe hypertension, hypokalemia, metabolic alkalosis, and hyporeninemia clinically. It is an inherited form of salt-sensitive hypertension [56, 84, 85]. Capillary sodium channels located on the aldosterone-sensitive distal nephron (ASDN) also play a key role in the homeostasis of sodium homeostasis. Clinical studies have confirmed that Liddle syndrome is related to the gene mutation of ENaC-NEDD4L-proteasome. A clinical study showed that Nedd4L gene single nucleic acid polymorphisms (SNPs) were closely related to hypertension [35]. Abriel and colleagues evidenced the loss of the Nedd4 binding site in ENaC in Liddle syndrome through immunofluorescence and other methods [86]. In addition, knockout of Nedd4L protein from adult mouse renal tubules can lead to the accumulation of ENaC, which may lead to increased cell surface channels and sodium reabsorption in distal nephrons, and ultimately resulting in hypertension [86]. The human Nedd4L gene, especially the evolutionary new subtype I, is a candidate gene for hypertension [38]. Certain small-molecule compounds could destabilize cell-surface ENaC or enhance the Nedd4L activity in the kidney, thus being hopeful candidates for anti-hypertension agents [87].

Nedd4L and myocardial remodeling
Nedd4L and cardiac diseases. Cardiac hypertrophy and dilated cardiomyopathy, as a type of myocardial remodeling [88–90], is one of the risk factors for heart failure (HF) [91–93]. Up till now, heart failure has been the main leading cause of death in the cardiovascular field [94, 95]. Nedd4L is the key player not only responsible for the regulation of blood pressure but also for the electrolyte balance in vivo. Cardiac hypertrophy and significantly decreased cardiac function were detected in Nedd4L knockout mice fed with chronic high-salt diet [57]. It is known that the density and expression of sodium channels in vivo play an important role in the pathogenesis of cardiovascular diseases. Among the ion channels of the heart, sodium voltage-gated channel alpha-subunit 5 (SCN5A) encodes the $Na_V1.5$ protein
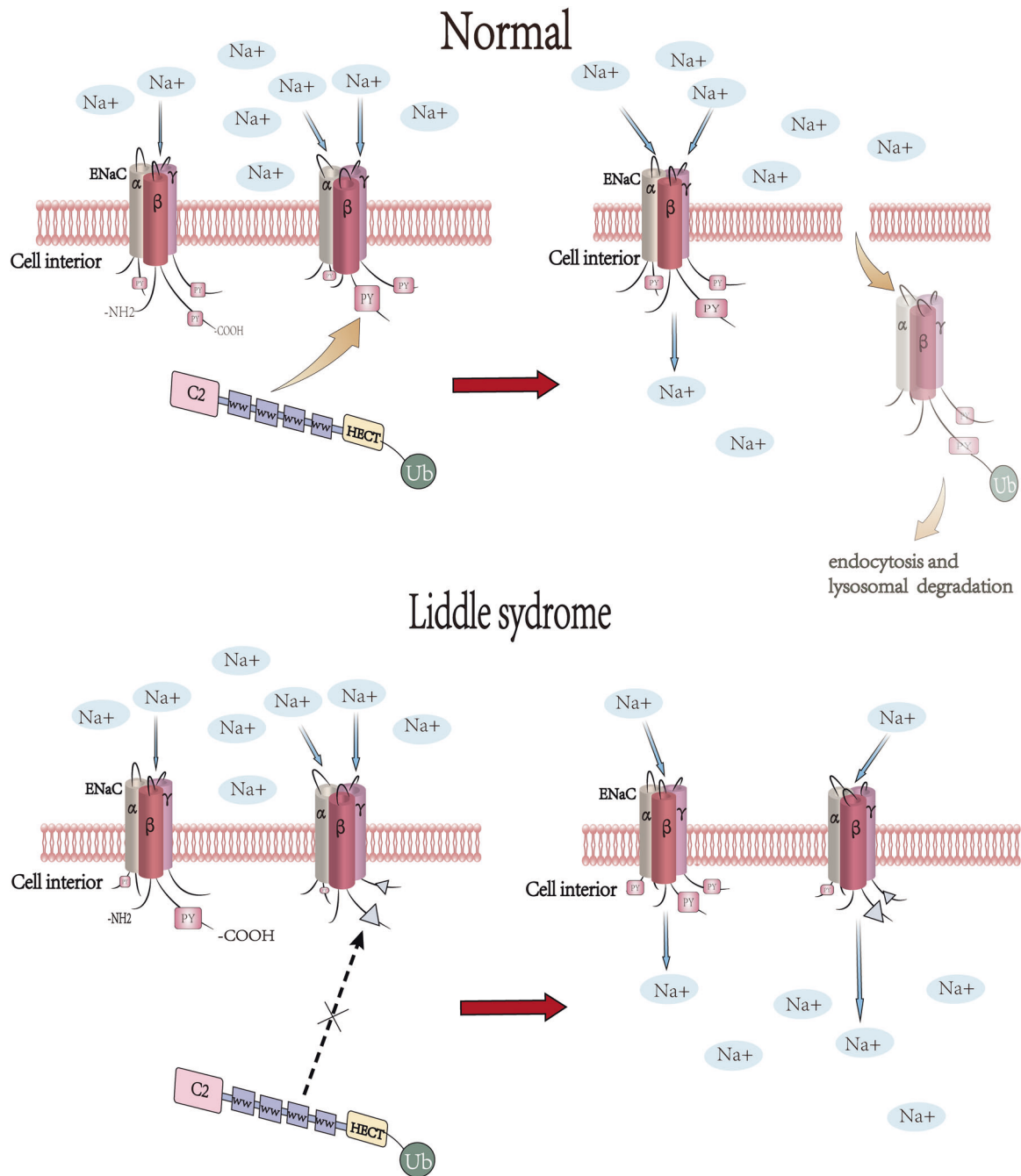
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Fig. 2 The molecular mechanism of the differences between the normal and liddle syndrome. Neuronal precursor cell expression developmentally downregulated 4-like(Nedd4L) is composed of C2 domain, four WW domains and HECT domain. α, β, and γ subunits of ENaC contains a conserved proline-tyrosine (PY) motif at the C-terminus which is also the binding site for the ubiquitin ligase Nedd4L. After mutation in this PY motif happens, the binding capacity to the WW domain of Nedd4L will decrease; thereby accelerate the cell activity and expression of ENaC, leading to its uncapable of degradation. Ultimately, increase the absorption of sodium ion in the distal nephron.

[96, 97]. As an essential transmembrane channel protein, Na$_V$1.5 is structurally composed of 4 similar domains, each of which contains 6 transmembrane proteins (S1-S6) linked by amino acid sequences [98]. The exact cause of dilated cardiomyopathy cannot always be found clinically, approximately 50 variants have been identified in patients with DCM that cause macroscopically dilated cardiomyopathy. This seems to imply that the occurrence of DCM is closely related to genetic background [99]. In 2008, Ge and his team conducted a research on a Chinese DCM pedigree and found that the cardiac sodium channel gene (SCN5A) A1180V was associated with the disease development in the whole family [97].

In basic researches, some scholars found that the mutation of SCN5A related to the occurrence of DCM is mainly distributed in the DI-S4 segment of the Nav1.5 protein, while the A1180V mutation occurs at DII-DIII segment [99, 100]. In fact, the electrophysiological damage associated with DCM is now thought to be caused by its variant which reduces peak sodium currents [99]. In addition, A1180V induces a 4.5mV negative inactivation offset of the mutant channel and also shows a slower recovery. This emergence of cardiac sodium ion current disturbance can greatly aggravate myocardial injury and ultimately lead to dilated cardiomyopathy, which in turn leads to heart failure [97]. Ge and

Table 1. This table shows how SCN5A, which encodes the NaV1.5 channel, plays a role in cardiomyopathies and arrhythmias (only includes well-studied diseases), most of which are associated with variants in SCN5A.

Mechanisms of the variant	Biological responses	References
The role of SCN5A gene encoding Nav1.5 protein in cardiovascular diseases (Only mention diseases for which there is a definite finding)		
Cardiomyopathy		
SCN5A-A1180V mutation, occurs at DII-DIII segment, induces a 4.5mV negative inactivation offset of the mutant channel and also shows a slower recovery.	Dilated cardiomyopathy (DCM)	[97, 99, 100]
Arrhythmia		
SCN5A variants may lead to the decreasing of cardiac excitability, resulting in the loss-of-function property of diminished electrical coupling between the sinoatrial node and surrounding atrial cells.	sick sinus syndrome (SSS)	[96, 124, 125]
The eight novel variants of SCN5A, including M138I, F428K, H445D, N470K, E655K, T113I, R1826C, V1951M, were all associated with the occurrence and susceptibility of AF.	Atrial fibrillation (AF)	[127, 128]
The decreased expression of atrial-specific gap junction protein connexin40 (Cx40); the mutations in the extremely important sodium ion channel gene SCN5A D1275N in the heart.	Atrial standstill (AS)	[129]
Brs is a heritable channelopathy. The SCN5A variants associated with BrS are usually located in the region between DI and DII. More than 300 SCN5A variants have been found to be associated with this inherited disease.	Brugada syndrome (Brs)	[133, 134]
SCN5A-p.Y1977N disrupts the common Nedd4L binding site (from PPxY to PPxN), thus prevent the process of ubiquitination.	Long QT syndrome	[135, 136]

his team also found that participants carrying the A1180V gene face higher risk for disease progression, and the mechanism might relate to changed sodium ion balance, resulting in electronic disorders and ultimately leading to structural heart disease [97, 101].

It is known that endocrine-related diseases such as diabetes [102–104] and insulin resistance [105] can also cause diabetic cardiomyopathy [106, 107]. The role of Nedd4L in the development of diabetic cardiomyopathy has also been explored in recent years. Nedd4L can become the phosphorylated form of Nedd4L, this process is mediated by SGK1. It acts as a direct downstream target of SGK1, and down-regulating SGK1 levels could down-regulate Nedd4L and vice versa. Shi et al. found that high glucose may downregulate the expression of SGK1, resulting in the reduction in the phosphorylated form of Nedd4L (an inactive state). This implies that SGK1 and Nedd4L are involved in the development of hyperglycemia-induced cardiomyopathy, future studies are warranted to validate whether targeting SGK1 and Nedd4L could provide a solution for the clinical treatment of diabetic cardiomyopathy or not [108].

Nedd4L and myocardial infarction. Myocardial infarction (MI) is related to the highest mortality rate among cardiovascular diseases [109–111]. Myocardial ischemia caused by MI, and heart remodeling [112] could be the causes of clinical death. Studies have shown that the loss of Nedd4L-C2 subtype can cause the cardiac conduction changes in resting state and responsible for the pro-arrhythmic change after acute myocardial infarction [113]. From a prognostic point of view, Huang and his colleagues found that the downregulation of circular RNA Nfix (circNfix) in cardiomyocytes can attenuate the process of myocardial remodeling after MI and partially restore myocardial functions. Y-box-binding protein 1 (Ybx1) [114] is an essential transcriptional protein in myocardial proliferation after myocardial infarction, which acts as a substrate for Nedd4L and can bind to Nedd4L for degradation. Studies have found that circNfix could bind Nedd4L. When circNfix is overexpressed, the binding of Nedd4L and circNfix can be promoted, resulting in the reduction of Ybx1. Downregulation of circNfix could upregulate Ybx1, promote proliferation and regeneration of cardiomyocytes, ultimately improve prognosis post-MI [115]. The in-depth understanding of the above mechanisms provides a theoretical basis for the clinical targeted therapy of patients with MI in the future, and also provides inspiration for new treatment approaches.

Heart failure which is a common heterogeneous clinical syndrome. Ubiquitination also played an important role in heart failure. Luo et.al. found that microenvironment change could lead to degradation changes of ENac by Nedd4L. When the intracellular concentration of calcium ion increases, the activity of Nedd4L increases, thereby promoting the degradation of the sodium ion channels located on the membrane. In addition, Nedd4L and its colocalization with sodium channels are also increased in case of heart failure [116]. It is also found that after the occurrence of HF, the expression of Nav1.5 protein was significantly downregulated [117]. A previous study demonstrated that miR-454 could affect heart failure (HF) progression by modulating Nedd4L/tropomyosin receptor kinase A (TrkA)/cyclic adenosine 3′,5′-monophosphate (cAMP) axis, in that miR-454 exerts anti-apoptotic and protective effects on cardiomyocytes by inhibiting Nedd4L, while Nedd4L stimulates the ubiquitination and degradation of TrkA protein. The miR-454 activates the cAMP pathway through the Nedd4L/TrkA axis, which ultimately inhibits cardiomyocyte apoptosis and alleviates myocardial injury. Results from this landmark study thus suggest that targeting the Nedd4L/TrkA/cAMP pathway may be a potential novel promising option for the prevention and treatment of heart failure [118].

Nedd4L and arrhythmias. The sodium channel has three subunits, α, β, and γ [56]. Sodium voltage-gated channel alpha-subunit 5 (SCN5A) (Table 1) encodes the voltage-dependent alpha-subunit of NaV1.5 protein [97, 98]. Nedd4L binds through its WW domain to the PY motif at the C-terminus of $Na_v1.5$ protein encoded by SCN5A [59, 119].This results in decreased protein expression and current through $Na_v1.5$ ubiquitination [113, 120]. The above can indicate that the mutation of the SCN5A gene encoding $Na_v1.5$ protein or the changes of its expression level are closely related to the occurrence of diseases with abnormal electrophysiological conduction under various factors [121]. Therefore, it is of importance to explore the mechanism of $Na_v1.5$ channel mutation for the prevention and treatment of arrhythmia, especially malignant arrhythmias [120, 122, 123].

In the field of the arrhythmia associated with the atrium, studies have found that mutations in SCN5A are associated with diseases such as sick sinus syndrome (SSS) [124]. Loss or enhancement of sodium channel function caused by SCN5A variants can lead to the decreasing of cardiac excitability, resulting in sinus node dysfunction. The most typical state caused by SCN5A variability is the loss of function of the sodium ion channel. In the molecular

Table 2. This table shows the roles and functions of Nedd4L in various cardiac diseases, especially hypertension.

Disease category	Involvement in the diseases	Functions and mechanisms	References
Roles and functions of Nedd4L in cardiac diseases			
Hypertension	Salt-sensitive hypertension	SGKI phosphorylates and inhibits Nedd4L which is a ubiquitin ligase, thereby failing to reduce the channel expression and stimulate ion channel degradation. The phosphorylation mediated by SGK1 induces the interaction of Nedd4L with members of the 14-3-3 protein family, which in turn disrupts the ubiquitination and degradation of ENaC by Nedd4L, ultimately leads to salt-sensitive hypertension.	[68, 69]
	Essential hypertension	Nedd4L isoform I can interact with other isoforms and can increase sodium reabsorption, which can lead to hypertension; the A allele of SNP rs4149601 of Nedd4L was associated with increased blood pressure, with the involvement of Nedd4L. The detailed mechanism may be that this allele could reduce the ubiquitination and degradation of epithelial sodium channels, resulting in an increase in the density of epithelial sodium channels or prolonged residence time on the cell surface, which will further lead to the increasing of the epithelial sodium transportation, eventually result in hypertension.	[38, 66]
	Liddle syndrome	Mutations in the β or α subunits of the PY motif in the ENaC lead to the binding capacity to the WW domain of Nedd4L decreased, thereby accelerate the cell activity of ENaC, promote apoptosis, increase the absorption of sodium ion and fluid in the distal nephron, and ultimately cause the blood volume and blood pressure elevated.	[56, 81–83]
Cardiomyopathy	Dilated cardiomyopathy	SCN5A-A1180V induces a 4.5mV negative inactivation offset of the mutant channel and also shows a slower recovery. This emergence of cardiac sodium ion current disturbance can greatly aggravate myocardial injury and ultimately lead to dilated cardiomyopathy.	[97]
	Diabetic cardiomyopathy	The miR-195-5p/SGK1/Nedd4L axis plays an important regulatory role in high glucose-induced cardiomyopathy.	[108]
	Myocardial infarction	When the circNfix is overexpressed in cardiomyocytes, it can promote the binding of Nedd4L and Ybxl, resulting in the reduction of Ybxl, hence leads to the induction of cardiomyocyte proliferation, which is not beneficial to prognosis.	[114, 115]
	Heart failure	The miR-454 activates the cAMP pathway through the NEDD4L/TrkA axis, which ultimately inhibits cardiomyocyte apoptosis and alleviates myocardial injury.	[118]
Arrhythmia	Long QT syndrome	SCN5A-p.Y1977N disrupts the common Nedd4L binding site (from PPxY to PPxN), thus prevents the process of ubiquitination.	[135, 136]

(Studies imply that there is not much direct connection between Nedd4L and arrhythmia, and most of the arrhythmias are related to the gene SCN5A that encodes the sodium channel. Shown in Table 1) Here, eight diseases are listed in the table.

level, it is manifested as the loss-of-function property of diminished electrical coupling between the sinoatrial node and surrounding atrial cells, resulting in outlet blocking, which is a common character in SSS [96, 125]. The other diseases most associated with SCN5A are currently found to be atrial fibrillation (AF) and atrial standstill. AF has always been the most common type of cardiovascular system disease in arrhythmia [126]. Clinically, its electrocardiogram often shows rapid irregular atrial fibrillation waves of 350–600 beats per minute. Experts have found that among patients diagnosed with AF, those with a family history of atrial fibrillation are more likely to develop new-onset AF [127], which implies that the occurrence of AF may be closely related to genetic factors [128]. In addition, studies have found that mutations or rare variants in the SCN5A gene may predispose people without underlying heart disease to AF. Hence, it seems that the susceptibility of AF is related to the variations in the gene named SCN5A which encoding the cardiac sodium channels [127]. However, AS is a rare arrhythmia [129]. In 2003, Groenewegen et al. investigated families with this rare disease background and found that the occurrence of AS was related to the decreased expression of atrial-specific gap junction protein connexin40 (Cx40), and the extremely important sodium ion channel gene SCN5A D1275N in the heart also has mutations. Hence the emergence of AS is also considered to appear under the joint influence of various factors [129].

Secondly, in the direction of arrhythmia related to the ventricle, there are also many arrhythmias related to the mutation of the sodium channel encoding gene SCN5A. Among the primary arrhythmic syndromes (including Brugada syndrome, long QT syndrome, short QT syndrome, early repolarization syndrome, etc.)

[130], Brugada syndrome (BrS) was found to be most associated with SCN5A [131]. In 1998, the variant of this gene encoding the sodium channel was discovered for the first time [132]. Eight allele-related inherited heart diseases have been found to be associated with the genetic mutations of SCN5A [133]. It has been found that the SCN5A variants associated with BrS are usually located in the region between DI and DII [134]. Currently, 21% of BrS probands are found to be carriers of SCN5A variants, and more than 300 SCN5A variants have been found to be associated with this inherited disease [134]. Other studies have found that the SCN5A-p.L1239P variant can enhance the ubiquitin and degradation of Na_V 1.5 channels due to the existence of a new binding site. Another variant, SCN5A-p.Y1977N, which disrupts the common Nedd4L binding site (from PPxY to PPxN), could prevent the process of ubiquitination, and this variant is associated with long QT syndrome [135, 136] (Table 2).

In addition, in a study of simulating myocardial infarction in Nedd4L-C2-KO mice, Minegishi et al. found that within 6 weeks after myocardial infarction in Nedd4L-C2-KO mice, the PR interval was significantly shortened compared with wild-type MI mice, QT interval and QTc was prolonged, T peak/T wave end interval showed an enhancement as well [113]. Experimental animal studies also showed that Nedd4L-C2-KO mice exhibited signs of bradycardia, QRS prolongation, QT interval prolongation, and suppressed PR interval in the resting state [113].

CONCLUSION
In conclusion, Nedd4L, as the dominating E3 ligase of the Nedd4 family, and related signaling play fundamental roles in

cardiovascular diseases. In this present review, we've already known that the ubiquitination ligase, Nedd4L, participates in various pathophysiological processes of cardiovascular diseases. The Nedd4L isoform is expressed in several organs, thus plays a key role in the post-transcriptional modification of sodium transporters and cardiac ion channels. The encoding gene SCN5A, ENaC, and the mediator SGK1 and etc. participate in the pathogenesis of various cardiac diseases. Either mutations or deletion of above genes could lead to the poor outcome. Several signaling pathways play important roles in regulating the body's blood pressure, among them, NEDD4L is a key regulator of these processes. Therefore, understanding their roles and targeting these molecules might hint novel therapeutic approaches to various cardiovascular diseases.

DATA AVAILABILITY

The [DATA TYPE] data used to support the findings of this study are available from the corresponding author upon request.

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